From the INTERNATIONAL BUREAU

# PCT

## NOTIFICATION OF ELECTION

(PCT Rule 61.2)

To:

Assistant Commissioner for Patents
United States Patent and Trademark Office
Box PCT
Washington, D.C.20231
ETATS-UNIS D'AMERIQUE

in its capacity as elected Office

| | |
|---|---|
| **Date of mailing** (day/month/year)<br>22 May 2000 (22.05.00) | |
| **International application No.**<br>PCT/EP99/08031 | **Applicant's or agent's file reference**<br>WOB 98BAINSS |
| **International filing date** (day/month/year)<br>22 October 1999 (22.10.99) | **Priority date** (day/month/year)<br>23 October 1998 (23.10.98) |
| **Applicant**<br>GESTIN, Jean-François et al | |

1. The designated Office is hereby notified of its election made:

[X] in the demand filed with the International Preliminary Examining Authority on:

27 April 2000 (27.04.00)

[ ] in a notice effecting later election filed with the International Bureau on:

2. The election [X] was

[ ] was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

| | |
|---|---|
| **The International Bureau of WIPO**<br>**34, chemin des Colombettes**<br>**1211 Geneva 20, Switzerland** | Authorized officer<br>F. Baechler |
| Facsimile No.: (41-22) 740.14.35 | Telephone No.: (41-22) 338.83.38 |

Form PCT/IB/331 (July 1992)

EP9908031

PATENT COOPERATION TREATY

PCT

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

| Applicant's or agent's file reference | FOR FURTHER ACTION | See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416) |
|---|---|---|
| WOB 98BAINSS | | |
| International application No. | International filing date *(day/month/year)* | Priority date *(day/month/year)* |
| PCT/EP99/08031 | 22/10/1999 | 23/10/1998 |
| International Patent Classification (IPC) or national classification and IPC<br>C07F9/38 | | |
| Applicant<br>INSTITUT NATIONAL DE LA SANTE ET DE LA....et al. | | |

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 8 sheets, including this cover sheet.

   ☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the report
   - II ☐ Priority
   - III ☒ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations suporting such statement
   - VI ☒ Certain documents cited
   - VII ☒ Certain defects in the international application
   - VIII ☒ Certain observations on the international application

| Date of submission of the demand | Date of completion of this report |
|---|---|
| 27/04/2000 | 12.01.2001 |
| Name and mailing address of the international preliminary examining authority:<br>European Patent Office<br>D-80298 Munich<br>Tel. +49 89 2399 - 0 Tx: 523656 epmu d<br>Fax: +49 89 2399 - 4465 | Authorized officer<br>Mitchell, G<br>Telephone No. +49 89 2399 8678 |

# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP99/08031

## I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments (Rules 70.16 and 70.17).)*:
**Description, pages:**

1-29 as originally filed

**Claims, No.:**

1-18 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

*(Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)*

6. Additional observations, if necessary:

**III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non-obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application.

☒ claims Nos. 13, 17, 18.

because:

☒ the said international application, or the said claims Nos. relate to the following subject matter which does not require an international preliminary examination (*specify*):
**see separate sheet**

☐ the description, claims or drawings (*indicate particular elements below*) or said claims Nos. are so unclear that no meaningful opinion could be formed (*specify*):

☐ the claims, or said claims Nos. are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for the said claims Nos. .

2. A meaningful international preliminary examination report cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

| | | | |
|---|---|---|---|
| Novelty (N) | Yes: | Claims | 1-17 |
| | No: | Claims | 18 |
| Inventive step (IS) | Yes: | Claims | |
| | No: | Claims | 1-18 |
| Industrial applicability (IA) | Yes: | Claims | 1-12, 15, 16 |

No: Claims

2. Citations and explanations
**see separate sheet**

## VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

**see separate sheet**

## VII. Certain defects in the international application

The following defects in the form or contents of the international application have been noted:
**see separate sheet**

## VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:
**see separate sheet**

**Re Item III**

**Non-establishment of opinion with regard to novelty, inventive step and industrial applicability**

Claims 13, 17 and 18 relate to subject-matter considered by this Authority to be covered by the provisions of Rule 67.1(iv) PCT. Consequently, no opinion will be formulated with respect to the industrial applicability of the subject-matter of these claims (Article 34(4)(a)(i) PCT).

For the assessment of the present claims 13, 17 and 18 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

**Re Item V**

**Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

The following documents are referred to:

D1: US 5 292 938
D2: J.Chem. Soc., Perkin Trans. 1, 1998, (2), pp 237-242, Loussquarn A et al.
D3: Inorg. Chem., vol 35 (21), pp. 6343-6348, Brechbiel M W et al.
D4: US 5 089 663

D4 refers to the semi-rigid chelate cyclohexyl EDTA which is "known and has been used in the preparation of copper complexes" (page 1, column 2, line 38-40). Therefore, claim 18, is a use claim which includes the use of CDTPA and CTTHA, (both of which are disclaimed in claim 1) is not new.

The technical problem addressed by the application is the provision of compounds of the formula I which can be complexed with $\alpha$, $\beta$ and $\gamma$ emmitter radiometals to form

complexes which have applications in radioimmunotherapy.

The prior art D1-D4 disclose compounds which are based on EDTA and which incorporate a cyclohexanic ring into the EDTA skeleton. The prior art disclose the use of these type of compounds as chelating agents that have a high affinity for metals in biological environments.

Claim 1 is defined by the use of a disclaimer; a disclaimer should only be used when the claim's remaining subject-matter cannot be defined more clearly and concisely by means of positive features. Moreover, the subject-matter of the disclaimer must be accidentally novelty destroying, that is to say that it should not relate to the same technical field as the application. No document has been cited in the present application to indicate the basis of the disclaimer used in claim 1, however, both CDTPA and CTTHA are well known in the field of the application. The structures of which are closely related to formula I of claim 1 of the present application, with the main difference being the nature of the substituent R; when R is anything but hydrogen.
D1 is directed to the preparation of new forms of CDTPA (column 4, line 5-10) and in particular CDTA (see column 4) wherein R is $NH_2$, -N=C=S, $-NHC(O)CH_2X$, which would render the solution of the technical problem addressed by the present application, as obvious to one skilled in the art.
D2, (page 238, column 2, line 65-67) also discloses 4-ICMP ((1R*,2R*, 4S*)-4-isothiocyanatocyclohexane-1,2-diamine-N,N,N',N'-tetrakisethanephosphonic acid), wherein R is an isocyanate function.
Since no unexpected technical effect has been demonstrated by either the compounds or complexes of the present application, no inventive step can be acknowledged as it would be obvious to one skilled in the art, from the teaching of the prior art, to derivatise the known complexes to arrive at analogues (Art. 33(3) PCT).

**Re Item VI**
**Certain documents cited**

D5: Mater. Sci. Forum, 1999, vol. 315-317, pp. 262-267, Loussquarn A et al.

The document D5 indicated on the international search report as a P-document will not be taken into consideration with regard to novelty or inventive step assessment. The

publication date of D5 falls in 1999. Since the priority document is not presently available, the priority is assumed to be valid. If, however, at the regional phase of examination, the priority is found to be invalid D5 may be used for novelty and inventive step assessment for the parts of the application for which priority is not valid.

**Re Item VII**

**Certain defects in the international application**

a) Terms such as "preferably" (claim 1), "in particular" (claim 4) and "more particularly" (claim 12 and 16), have no limiting effect on the scope of said claim i.e. the features following these expressions are regarded as entirely optional (see PCT Guidelines CIII, 4.6).

b) Since, in claim 4, the feature following "in particular" is that which differentiates the subject-matter of claims 4 and 5, this term should be deleted and appropriate amendments to claim 4 should be made, such that it claims different subject-matter to claim 5.

c) Contrary to the requirements of Rule 5.1(a)(ii) PCT, the relevant background art disclosed in the documents 1-4 are not mentioned in the description, nor are these documents identified therein.

**Re Item VIII**

**Certain observations on the international application**

a) The full terms for the abbreviations, CDTPA and CTTHA, used in claims 1 and 18 should be given for purposes of clarity.

b) Line 13 on page 2 of the description is unclear (Art. 5 PCT).

c) The term "all the coupling functions" is broad and renders the subject-matter of claim 4 unclear.

d) Claim 18 consists of four uses claims, which can be categorised as first medical use followed by second, third and fourth medical uses. These uses should be

formulated as individual use claims for purposes of clarity. However, it is pointed out that in order for a first medical use claim to be acceptable under PCT the known substance or composition must not have been disclosed for use in surgery, therapy or diagnostic methods practised on the human or animal body, i.e it's use must be new and inventive.

P[redacted]NT COOPERATION TREATY

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# INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

| Applicant's or agent's file reference | **FOR FURTHER ACTION** see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below. | |
|---|---|---|
| WOB 98BAINSS | | |
| International application No. | International filing date *(day/month/year)* | (Earliest) Priority Date *(day/month/year)* |
| PCT/EP 99/ 08031 | 22/10/1999 | 23/10/1998 |
| Applicant | | |
| INSTITUT NATIONAL DE LA SANTE ET DE LA....et al. | | |

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 3 sheets.

[X] It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

[ ] the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

[ ] contained in the international application in written form.

[ ] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readble form.

[ ] the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. [ ] **Certain claims were found unsearchable** (See Box I).

3. [ ] **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

[ ] the text is approved as submitted by the applicant.

[X] the text has been established by this Authority to read as follows:

CHELATING AGENTS FOR RADIOIMMUNOTHERAPY

5. With regard to the **abstract,**

[X] the text is approved as submitted by the applicant.

[ ] the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. ---

[ ] as suggested by the applicant.

[ ] because the applicant failed to suggest a figure.

[ ] because this figure better characterizes the invention.

[ ] None of the figures.

# INTERNATIONAL SEARCH REPORT

International Application No
PCT/[redacted] 99/08031

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07F9/38 C07C229/16 A61K51/10 A61K51/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07F C07C A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | US 5 292 938 A (RONNIE C. MEASE)<br>8 March 1994 (1994-03-08)<br>the whole document<br>---<br>-/-- | 1-18 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

| Date of the actual completion of the international search | Date of mailing of the international search report |
|---|---|
| 21 December 1999 | 11/01/2000 |

| Name and mailing address of the ISA | Authorized officer |
|---|---|
| European Patent Office, P.B. 5818 Patentlaan 2<br>NL - 2280 HV Rijswijk<br>Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,<br>Fax: (+31-70) 340-3016 | Beslier, L |

1

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication,where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | LOUSSOUARN A ET AL: "Synthesis of new bifunctional chelating agents: (1R*,2R*,4S*)-4-isothiocyanatocyclohexane-1,2-diamine-N,N,N',N'-tetra kismethanephosphonic acid (4-ICMP) and (1R*,2R*,4S*)-4-isothiocyanato cyclohexane-1,2-diamine-N,N,N',N'-tetrakis ethanephosphonic acid (4-ICEP)" J. CHEM. SOC., PERKIN TRANS. 1 (JCPRB4,0300922X);1998; (2); PP.237-242, - 21 January 1998 (1998-01-21) XP002097516 Institut de Biologie;INSERM U.463 (ex-U.211); Nantes; 44035; Fr. (FR) cited in the application the whole document | 1-18 |
| Y | BRECHBIEL M W ET AL: "Preparation of the Novel Chelating Agent N-(2-Aminoethyl)-trans-1,2 -diaminocyclohexane-N,N',N''-pentaacetic Acid (H5CyDTPA), a Preorganized Analog of Diethylenetriaminepentaacetic Acid (H5DTPA), and the Structures of BiIII(CyDTPA)2- and BiIII(H2DTPA) Complexes" INORG. CHEM. (INOCAJ,00201669); VOL.35 (21); PP.6343-6348, - 9 October 1996 (1996-10-09) XP002097517 National Institutes of Health;Chemistry Section; Bethesda; 20892; MD; USA (US) the whole document | 1-18 |
| Y | US 5 089 663 A (RONNIE C. MEASE) 18 February 1992 (1992-02-18) the whole document | 1-18 |
| P,X | LOUSSOUARN A ET AL: "Simple and general procedure for the synthesis of semi-rigid chelating agents for radiometal complexation studies and its application to semi-rigid functionalised ligands (BCA) synthesis" MATER. SCI. FORUM (MSFOEP,02555476);1999; VOL.315-317 (RARE EARTHS '98); PP.262-267, XP000865647 Institut Biologie;Nantes; F-44035; Fr. (FR) the whole document | 1-18 |

# INTERNATIONAL SEARCH REPORT

Informa patent family members

International Application No
PCT 99/08031

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| US 5292938 A | 08-03-1994 | US 5635157 A | 03-06-1997 |
| US 5089663 A | 18-02-1992 | US 5021571 A | 04-06-1991 |
| | | US 5334729 A | 02-08-1994 |

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International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C07F 9/38, C07C 229/16, A61K 51/10, 51/08

A1

(11) International Publication Number: WO 00/24751

(43) International Publication Date: 4 May 2000 (04.05.00)

(21) International Application Number: PCT/EP99/08031

(22) International Filing Date: 22 October 1999 (22.10.99)

(30) Priority Data:
98402648.4 23 October 1998 (23.10.98) EP

(71) Applicant *(for all designated States except US)*: I.N.S.E.R.M. (INSTITUT NATIONAL DE LA SANTE ET DE LA RECHERCHE MEDICALE) [FR/FR]; 101, rue de Tolbiac, F-75654 Paris Cedex 13 (FR).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: GESTIN, Jean-François [FR/FR]; 5, chemin de la Coulée, F-44470 Mauves-sur-Loire (FR). LOUSSOUARN, Anthony [FR/FR]; 53, rue Fauré, F-44000 Nantes (FR). FAIVRE-CHAUVET, Alain [FR/FR]; 24, rue E. Zola, F-44300 Reze (FR).

(74) Agents: DEMACHY, Charles et al.; Grosset-Fournier & Demachy, 20, rue de Maubeuge, F-75009 Paris (FR).

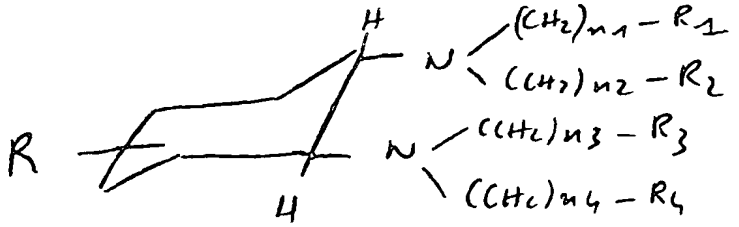
(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
*With international search report.*

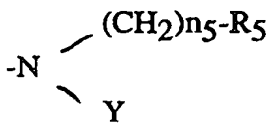
(54) Title: CHELATING AGENTS FOR RADIOIMMUNOTHERAPY

(57) Abstract

The invention relates to compounds of formula (I): in which: $n_1$, $n_2$, $n_3$ and $n_4$, represent an integer from 1 to 5, $R_1$, $R_2$, $R_3$ and $R_4$, independently from each other, represent -COOH, $-PO(OH)_2$, at least one of $R_1$, $R_2$, $R_3$ or $R_4$ represents a group (II), whercin $n_5$ represents an integer from 1 to 5, $R_5$ represents -COOH or $-PO(OH)_2$, and Y represents H or a group $-(CH2)n_6-R_6$ in which $n_6$ represents an integer from 1 to 5, and $R_6$ represents -COOH or $-PO(OH)_2$, R represents H, or a group carrying a function linked to molecules able to bind with epitopes at the surface of cells. The invention also relates to the processes of preparation of said compounds, and to their use in pharmaceutical compositions and in diagnosis methods.



(I)



(II)

*FOR THE PURPOSES OF INFORMATION ONLY*

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

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# CHELATING AGENTS FOR RADIOIMMUNOTHERAPY

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The invention relates to compounds useful as chelating agents, complexes between said compounds and radioelements, and to their uses, in particular in pharmaceutical compositions and compositions for the diagnosis of pathologies such as cancers.

Immunotherapy with radiolabeled antibodies should allow fairly specific targeting of certain cancers (Schubiger et al., 1996; Parker, 1990). However, iodine-131 (Bardies et al., 1992; Stein et al., 1995) may not be the best isotope for tumor therapy because of its limited specific activity, low beta-energy, relatively long half-life and strong gamma emission.

Another approach to improving therapeutic efficacy is the use of replacement isotopes with better physical properties. Chelators that can hold radiometals with high stability under physiological conditions are essential to avoid excessive radiation damage to non-target cells. Moreover, the development of new bifunctional chelating agents is essential for this purpose. Thus synthesis of new chelating agents able to bind radiometals such as rhenium-188, yttrium-90, samarium-153 or Bismuth-213 and in general all the $\alpha$ and $\beta$ particles emitters will be required to possess sufficiently stable chelators.

Accordingly, one of the aim of the invention is to provide chelating agents forming stable complexes *in vivo* with the numerous potential candidates for such applications.

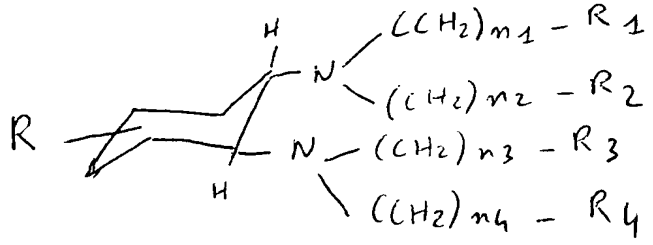
The stability of a non-macrocyclic ligand can be favourably influenced by the preorganization of the open chain. In fact, a semi-rigid structure such as that of *trans* 1-2 diaminocyclohexane limits the rotation of the ethylene bridge, so that the purpose of the cyclohexane design is to preorient the four pendent arms in a skew position.

A first investigation (Mease et al., 1990), which was guided by a study performed on polyaminocarboxylic acid ligands incorporating the skeleton of ethylenediaminetetraacetic acid (EDTA) in a cyclohexane structure, showed the influence of this semi-rigid structure on the stability of the resulting complexes. A second study (Goeckeler et al., 1987) of the stability of lanthanides as $^{153}$Sm-polyaminophosphonic acid complexes showed that ethylenediamine tetramethylphosphonic acid (EDTMP) derivatives allow stable quantitative $^{153}$Sm chelation.

The (1*R**, 2*R**, 4*S**)-4-acetamido-1,2-diaminocyclohexane dihydro chloride compound, the structural derivative of *trans*-1,2-diaminocyclohexane, have been prepared (Gestin et al., 1997; Loussouarn, et al., 1998). This intermediate, which is functionalized at position 4 of the cycle by a protected amine termination (Meares et al., 1984) for future covalent attachment to biomolecules, allows the introduction of different chelating groups via the free amines.

The Inventors have developed a new simple and efficient synthesis pathway from *trans* -1,2- diaminocyclohexane to provide access to a new class of semi-rigid chelating agents. This same reactional scheme applies to the reactional intermediary, (1*R**, 2*R**, 4*S**)-4-acetamido-1,2-diaminocyclohexane dihydrochloride, which allows the synthesis of these same chelating agents, though functionalized back of the cycle by a termination allowed coupling to an antibody or any other biological substance such as a hapten.

The present invention relates to compounds of the following formula (I) :



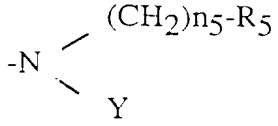

(I)

in which :

- $n_1$, $n_2$, $n_3$ and $n_4$, independently from each other, represent an integer from 1 to 5, preferably from 1 to 3,
- $R_1$, $R_2$, $R_3$ and $R_4$, independently from each other, represent :
  - . –COOH,
  - . $-PO(OH)_2$,
  - $-N\begin{cases}(CH_2)n_5-R_5\\ Y\end{cases}$

wherein $n_5$ represents an integer from 1 to 5, preferably from 1 to 3, $R_5$ represents –COOH or $-PO(OH)_2$, and Y represents H or a group $-(CH2)n_6-R_6$ in which $n_6$ represents an integer from 1 to 5, preferably from 1 to 3, and $R_6$ represents –COOH or $-PO(OH)_2$,

provided that at least one of $R_1$, $R_2$, $R_3$ or $R_4$ represents a group



such as defined above,

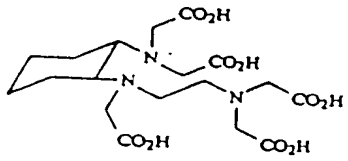
- R represents :

. H, or $-NHCOCH_3$, or

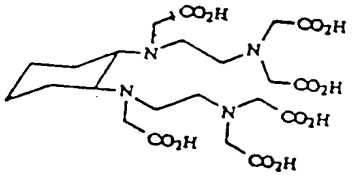
. a group carrying a function liable to bind, if necessary via a binding site, to molecules, such as antibodies, haptens or peptides, which are able to bind specifically with epitopes located at the surface of the cells of the organism, or to chemical or biological compounds located at the surface of a solid carrier, or

. a group carrying a function linked, if necessary via a binding site, to molecules as defined above,

the two following compounds, CDTPA and CTTHA, being excluded :



CDTPA

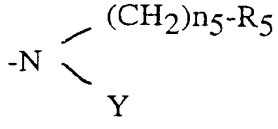


CTTHA

The invention relates more particularly to compounds of formula (I) such as defined above, characterized in that :

- when $R_1$, $R_2$, $R_3$ or $R_4$ represents -COOH or $-PO(OH)_2$, then $n_1$, $n_2$, $n_3$ or $n_4$ represents 1 respectively,

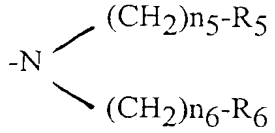
- when $R_1$, $R_2$, $R_3$ or $R_4$ represents a group



then $n_1$, $n_2$, $n_3$ or $n_4$ represents 2 or 3 respectively, and preferably 2,

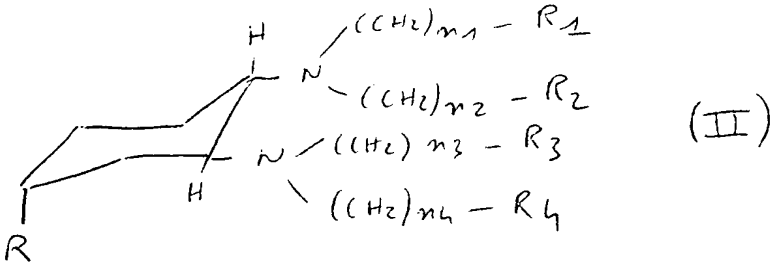
- $n_5$, and optionally $n_6$, represents 1.

The invention also relates more particularly to compounds of formula (I) such as defined above, characterized in that at least one, and more preferably two of $R_1$, $R_2$, $R_3$ and $R_4$, represent a group



wherein $n_5$, $n_6$, $R_5$ and $R_6$ are defined above.

Preferred compounds of formula (I) such as defined above, wherein R is different from hydrogen, are compounds of the following formula (II) :



(II)

wherein $n_1$, $n_2$, $n_3$, $n_4$, $R_1$, $R_2$, $R_3$, $R_4$ and R are such as defined above.

The invention relates more particularly to compounds of formula (I) or (II) such as defined above, characterized in that R represents a group carrying a function liable to bind, if necessary via a binding site, to molecules, such as antibodies, haptens or peptides, as defined above, and in particular R represents a group chosen among all the coupling functions for vector or solid support binding, such as the following groups :

. alcohol group, such as -OH,

. amino group, such as $-NH_2$, $-NO_2$,

. aldehyde group, such as -CHO,

. carboxylic group, such as -COOH,

. anhydride group, such as -CO-O-CO-R",

. $-CO-CH_2X$, wherein X represents an halogen atom, such as Cl or Br,

. -CO-X, wherein X represents an halogen atom, such as Cl or Br,

. a diazonium ion $N_2^+$,

. an activated ester, such as -COOR", R" = ethyl or N-hydrosuccinimide,

. a sulfonic group, such as $SO_3H$,

. a thiocyanate group, such as -NCS, or an isocyanate -NCO, or a -NH-NCS group

. a thiol group, such as -SH,

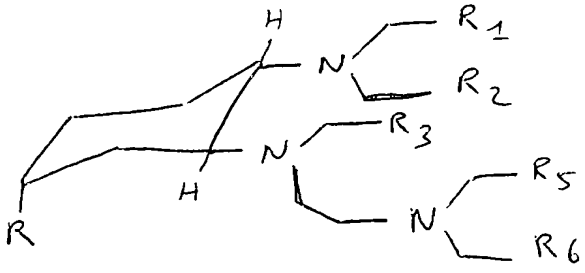
. a disulfure group, such as -S-S-R".

The invention also concerns compounds of formula (I) or (II) such as defined above, characterized in that R represents a group carrying a function linked, if necessary via a binding site, to molecules, such as antibodies, haptens or peptides, as defined above, and more particularly R represents a group chosen among the following groups :

. -O-CO-R',
. -NH-CO-R',
. -NH-CS-R',
. -CH=N-R',
. -CO-NH-R',
. -CO-$CH_2$-NH-R',
. -N=N-NH-R',
. -$SO_2$-NH-R',
. -NH-CS-NH-R',
. -thioether-R',
. -CO-S-R',
. -CO-$CH_2$-S-R',
. -S-S-R',
. -NH-$CH_2$-R',
. -CO-NH-N=CH-R',
. -CS-NH-N=CH-R',

wherein R' represents said molecule.

The invention concerns more specifically compounds such as described above of the following formula (III) :



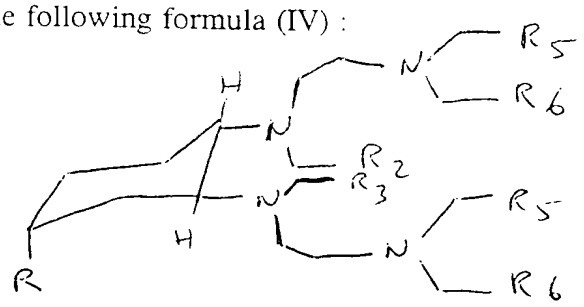

(III)

in which $R_1$, $R_2$, $R_3$, $R_5$ and $R_6$ independently from each other represent -COOH or -$PO(OH)_2$, and R is a group as defined above.

Preferred compounds of formula (III) are such that :

. $R_1 = R_5 = R_6 = COOH$ and $R_2, = R_3 = PO(HO)_2$, or
. $R_1 = R_2 = R_3 = R_5 = R_6 = COOH$, or
. $R_1 = R_2 = R_3 = R_5 = R_6 = PO(OH)_2$.

The invention also concerns more specifically compounds such as described above, of the following formula (IV) :



(IV)

wherein $R_2$, $R_5$ and $R_6$, independently form each other, represent -COOH or -$PO(OH)_2$, and R is a group as defined above.

Preferred compounds of formula (IV) are such that :

. $R_2 = R_3 = PO(OH)_2$, and $R_5 = R_6 = COOH$, or

. $R_2 = R_3 = R_5 = R_6 = COOH$, or

. $R_2 = R_3 = R_5 = R_6 = PO(OH)_2$.

The invention also relates to complexes between a compound such as described above, and a radioactive element, said complexes resulting from the association of said radioelement with the -COOH and/or -$PO(OH)_2$ groups of said compound (the bonds between said radioelement and said compound being ionic bonds).

The above-mentioned radioelements are more particularly $\alpha$, $\beta$ or $\gamma$ emitter radiometals, and preferably from the groups of actinides or lanthanides.

The invention relates more particularly to complexes such as described above, characterized in that said radioelements are $\alpha$ or $\beta$ emitter radiometals (susceptible to be used in therapy, and more particularly in radioimmunotherapy in the frame of cancer treatments).

Advantageously, $\alpha$ emitter radiometals are chosen among the followings : Actinium 225, Bismuth 213.

Advantageously, $\beta$ emitter radiometals are chosen among the followings : $^{33}P$, $^{199}Au$, $^{121}Sn$, $^{177}Lu$, $^{67}Cu$, $^{105}Rh$, $^{47}Sc$, $^{77}As$, $^{153}Sm$, $^{159}Gd$, $^{143}Pr$, $^{186}Re$, $^{111}Ag$, $^{149}Pm$, $^{109}Pd$, $^{166}Ho$, $^{32}P$, $^{188}Re$, $^{194}Ir$, $^{142}Pr$, $^{90}Y$.

Preferred complexes with radiometals used in therapy, as defined above, are such that the compound is chosen among those wherein R represents a group carrying a function linked, if necessary via a binding site, to molecules as defined above, and more particularly among those compounds wherein the group R comprises :

- an antibody (polyclonal or monoclonal) liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism,

- or an hapten, i.e. a non-immunogenic molecule of low MW capable of inducing the production of antibodies against itself, said hapten being liable to recognize and to bind to one or several molecules already bound (in a first step of the treatment) to epitopes on the surface of specific cells in the organism,

- or a peptide resulting from the association of different amino acids and liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism.

The invention also concerns the use of a complex such as described above, for the manufacture of a medicament for radioimmunotherapy (also called radiopharmaceutical), in particular for the treatment of cancers, or for the treatment against metastase proliferation.

More particularly, the invention relates to the use of a complex such as defined above, for the manufacture of a medicament for the treatment of :

- lung cancer, said complex preferably being such that it comprises a radioelement chosen among : $^{188}Re$, $^{186}Re$, $^{153}Sm$, $^{67}Cu$ and $^{90}Y$, and wherein R comprises an antibody specific for lung cancer cells, such as Anti N-CAM Antibody, Anti CEA Antibody, Anti Carbohydrates Antibodies, or an hapten chosen among Anti N-CAM-679 Bispecific antibody, Anti CEA-679 Bispecific antibody, Anti Carbohydrates-679 Bispecific antibody, Anti N-CAM-734 Bispecific antibody, Anti CEA-734 Bispecific antibody, Anti Carbohydrates-734 Bispecific antibody,

- liver and pancreatic cancers, said complex preferably being such that it comprises a radioelement chosen among those cited above in the case of lung cancer, and wherein R comprises an antibody specific for liver and pancreatic cancer cells, such as antibodies and haptens described above in the case of lung cancer,

- ovarian cancer, said complex preferably being such that it comprises a radioelement chosen among those cited above in the case of lung cancer, and wherein R comprises an antibody specific for ovarian cancer cells, such as OC125, MOV18, MOV19, OVTL3, or an hapten chosen among OC125-679 Bispecific antibody, MOV18-679 Bispecific antibody, MOV19-679 Bispecific antibody, OVTL3-679 Bispecific antibody, OC125-734 Bispecific antibody, MOV18-734 Bispecific antibody, MOV19-734 Bispecific antibody, OVTL3-734 Bispecific antibody,

- bladder cancer, said complex preferably being such that it comprises a radioelement chosen among those cited above in the case of lung cancer, and

wherein R comprises an antibody specific for bladder cancer cells, such as AC48-127, or an hapten chosen among 48-127 Bispecific antibody, 48-127-679 Bispecific antibody, 48-127-734 Bispecific antibody,

- colorectal cancer, said complex preferably being such that it comprises a radioelement chosen among those cited above in the case of lung cancer, and wherein R comprises an antibody specific for colorectal cancer cells, such as Anti CEA Antibody, Anti Carbohydrates Antibodies, or an hapten chosen among Anti CEA-679 Bispecific antibody, Anti Carbohydrates-679 Bispecific antibody, Anti CEA-734 Bispecific antibody, Anti Carbohydrates-734 Bispecific antibody,

- thyroïd medullary cancer, said complex preferably being such that it comprises a radioelement chosen among those cited above in the case of lung cancer, and wherein R comprises an antibody specific for thyroïd medullary cancer cells, such as Anti CEA Antibody, or an hapten chosen among Anti CEA-679 Bispecific antibody, Anti CEA-734 Bispecific antibody,

- lymphoma, said complex preferably being such that it comprises a radioelement chosen among : $^{213}Bi$, $^{225}Ac$, $^{153}Sm$, and $^{67}Cu$, and wherein R comprises an antibody specific for lymphoma cells, such as specific antibody against expressed antigens surfaces lymphocyte cells, e.g. CD19, CD37, or an hapten such as bispecific antibody against expressed antigens surfaces lymphocyte cells, e.g. CD19-679, CD37-679, CD19-734, CD37-734,

- myeloma, said complex preferably being such that it comprises a radioelement chosen among : $^{213}Bi$, $^{225}Ac$, $^{153}Sm$, and $^{67}Cu$, and wherein R comprises an antibody specific for myeloma cells, such as specific antibody against expressed antigens surfaces myeloma cells, e.g. BB4, or an hapten such as bispecific antibody against expressed antigens surfaces myeloma cells, BB4-679, BB4-734,

- osteoarticular pathology, particularly in bone cancer extension balance.

The invention also concerns pharmaceutical compositions characterized in that they comprise an effective amount of a complex such as described above, in association with a suitable pharmaceutical carrier.

Pharmaceutical compositions according to the invention are more particularly characterized in that they are in a form suitable for an IV or IP administration in located areas.

Preferred pharmaceutical compositions according to the invention, are characterized in that the daily dosage is comprised between 1 and 100MBq /kg, e.g. between 3,7 and 74MBq/kg.

The invention also relates to complexes, as defined above, between a compound such as described above, and a radioactive element, characterized in that the radioelements are γ emitter radiometals (i.e. radiometals susceptible to be used in diagnosis methods, such as radioimmunoscintigraphy).

Advantageously, said radiometals arechosen among $^{111}In$, $^{99m}Tc$, $^{64}Cu$.

Preferred complexes with radiometals used in diagnosis, as defined above, are such that the compound is chosen among those wherein R represents a group carrying a function linked, if necessary via a binding site, to molecules as defined above, and more particularly among those compounds wherein the group R comprises :

- an antibody (polyclonal or monoclonal) liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism,

- or an hapten, i.e. a non-immunogenic molecule of low MW capable of inducing the production of antibodies against itself, said hapten being liable to recognize and to bind to one or several molecules already bound (in a first step of the method of diagnosis) to epitopes on the surface of specific cells in the organism,

- or a peptide resulting from the association of different amino acids and liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism.

The invention also concerns the use of a complex such as described above, for carrying out diagnosis methods such as radioimmunoscintigraphy.

More particularly, the invention concerns the use of a complex such as defined above, for carrying out the following diagnosis methods by radioimmunoscintigraphy :

- diagnosis of cancers, such as cited above, the complex used being preferably such that it comprises $^{111}In$, or $^{99m}Tc$ as radioelements, and R comprises an antibody or a hapten specific for such cancer cells, such as antibodies or haptens mentioned above in the frame of the cancers listed above,

- diagnosis of cardiovascular diseases, such as graft rejection, myocardic infarcts,

- diagnosis of cerebral diseases,

- diagnosis of renal diseases, in paricular in the study of individual kidney functions, location of ectopic kidney, renal filtration and secretion troubles,

- vascular diseases, such as embolism and thrombosis, the complex used being preferably such that it comprises $^{111}In$, or $^{99m}Tc$ as radioelements, and R comprises an antibody such as anti platelets or anti fibrin antibodies.

The invention also concerns the use of the complexes defined above for carrying out bone scintigraphy, in particular in the frame of the diagnosis of osteoarticular pathology, particularly in bone cancer extension balance.

The invention also relates to methods for the *in vitro* diagnosis of pathologies listed above, characterized in that it comprises the steps of incubating a biological sample, such as serum, plasma urines, with a complex as described above, the components of the biological sample being fixed to a solid carrier, rinsing the solid carrier and detecting the $\gamma$ emission of the complex bound to the components of the sample on the solid carrier.

The invention also concerns the kits for carrying out said diagnosis methods, said kits comprising complexes as described above according to the invention.

The invention also relates to the use of a compound of formula (I) defined above, included compounds CDTPA and CTTHA, for the manufacture of a medicament for the treatment of pathologies where ionic imbalances occur, or against the formation of stones in the organism.

The invention also relates to the use of a compound of formula (I) defined above, included compounds CDTPA and CTTHA, for carrying out a process for the detoxication of polluted medium, such as liquid phases polluted by bivalent or trivalent metals radioactives or not.

The invention also concerns a process for the detoxication of a polluted medium comprising the steps of contacting said medium with a compound as defined above, advantageously itself bound to a solid carrier, and recovering said medium substantially free of contaminants which are bound to said compound on the solid carrier.

The invention also relates to the use of a compound of formula (I) defined above, included compounds CDTPA and CTTHA, for carrying out a process for the radionuclides purification, said compound of the invention being bound to a solid phase.

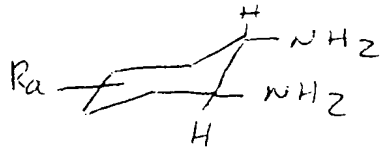
The invention also relates to the use of a complex between of formula (I) defined above, included compounds CDTPA and CTTHA, for carrying out a bone scintigraphy, in particular in the frame of the diagnosis of osteoarticular pathology, particularly in bone cancer extension balance.

The invention also relates to processes for preparing compounds and complexes as described above.

A process for the preparation of compounds according to the invention, comprises the following steps :

The invention also relates to processes for preparing compounds and complexes as described above. A process for the preparation of compounds according to the invention, comprises the following steps :

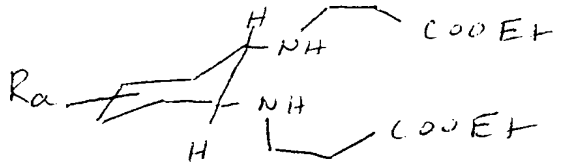
- contacting trans-1,2-diaminocyclohexane of the following formula a :



a

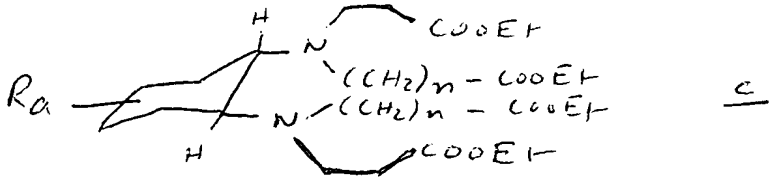
wherein $R_a$ is H or $NHCOCH_3$,

* either with vinyl propionate, preferably by stirring 20h at room temperature, leading to the following compound b



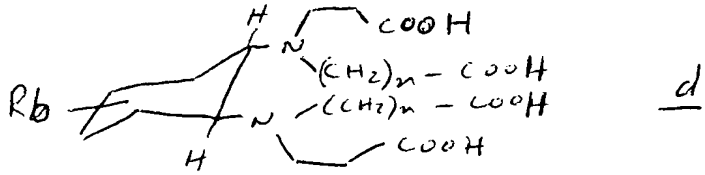
b

. contacting compound b with X-$(CH_2)_n$-COOEt, wherein X represents an halogen atom, and n represents an integer from 1 to 5, preferably at reflux during 15h, leading to the following compound c



c

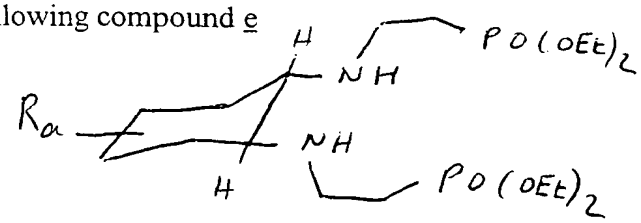
. treating compound c with HCl, preferably 6N HCl at reflux overnight, leading to the following compound d



d

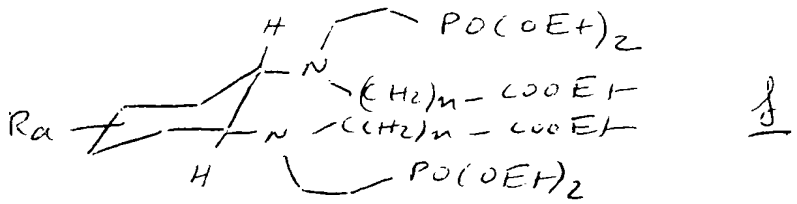
wherein $R_b$ represents H or $NH_2$,

* or with diethyl vinyl phosphonate, preferably by stirring 15h at reflux, leading to the following compound e



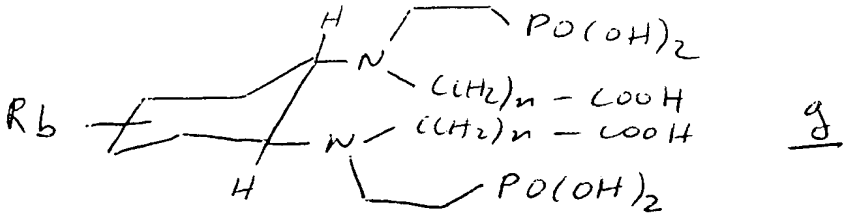
e

. contacting compound e with X-$(CH_2)_n$-COOEt, wherein X represents an halogen atom, and n represents an integer from 1 to 5, preferably at reflux during 15h, leading to the following compound f



f

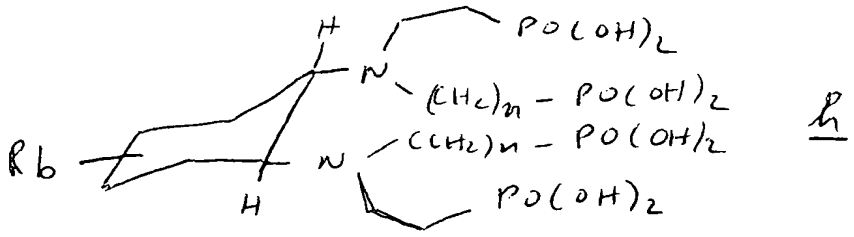
. treating compound f with HCl, preferably 6N HCl at reflux overnight, leading to the following compound g



g

wherein $R_b$ represents H or $NH_2$,

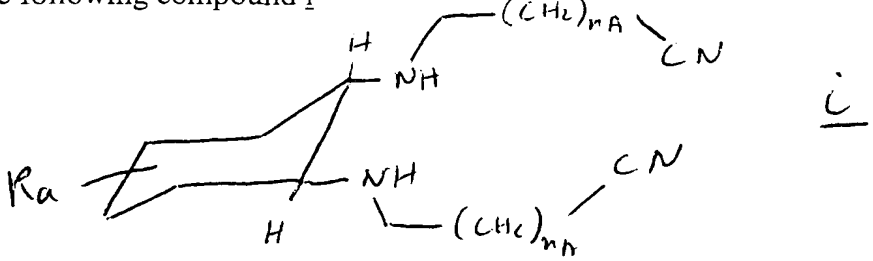
. if desired, treating compound g with phosphorous acid, preferably by stirring 30 mn at 80°C, leading to the following compound h



h

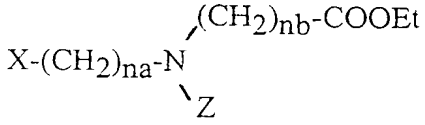
Another process for the preparation of compounds according to the invention, comprises the following steps :

- contacting the compound of formula a described above with a compound of formula $H_2C$=CH-$(CH_2)_{nA}$-CN wherein nA = 0 (acrylonitrile), or nA represents a integer from 1 to 3, preferably at room temperature during 20h, leading to the following compound i

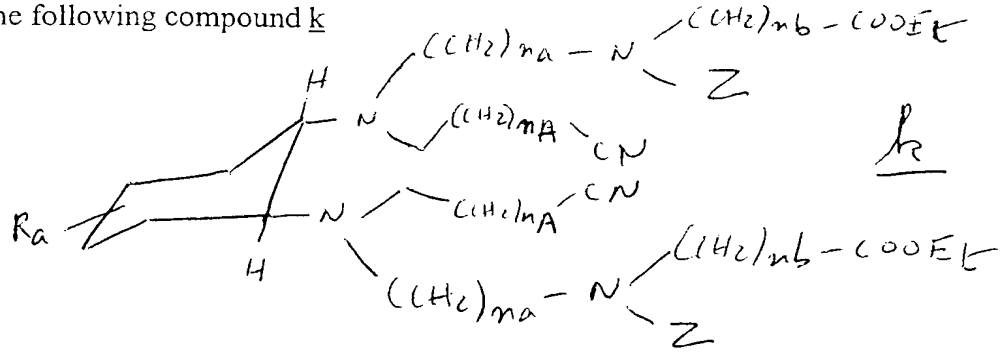


i

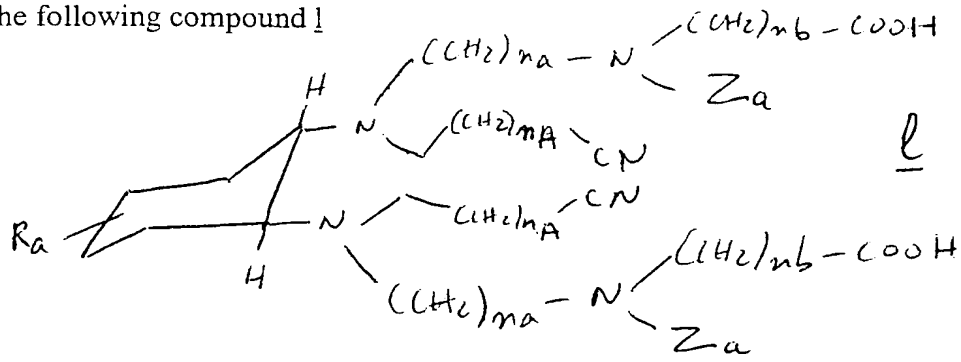
- contacting compound i with the following compound j



wherein X represents an halogen atom, na and nb, independently from each other represent an integer from 1 to 5, Z represents H or $(CH_2)_{nc}$-COOEt, and nc represents an integer from 1 to 5, preferably at 70°C during 2 days, leading to the following compound k



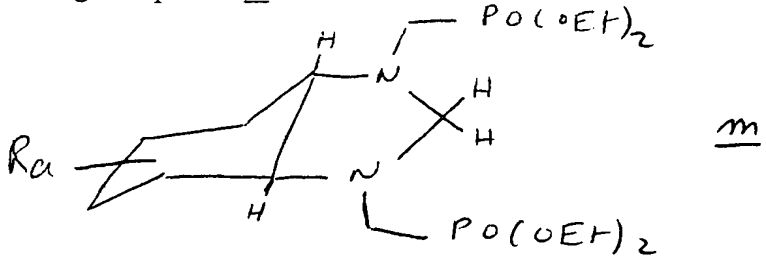
. treating compound k with HCl, preferably 6N HCl at reflux overnight, leading to the following compound l



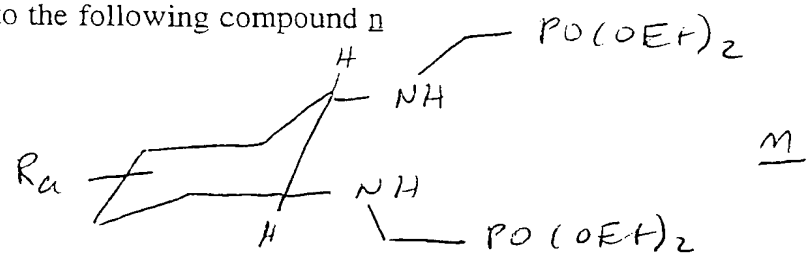
wherein Za represents H or -(CH2)nb-COOH, nA, na and nb being such as defined above, and $R_b$ represents H or $NH_2$.

Another process for the preparation of compounds according to the invention, comprises the following steps :

- contacting the compound of formula a described above with paraformaldehyde and diethylphosphite, preferably in THF at reflux during 4h, leading to the following compound m



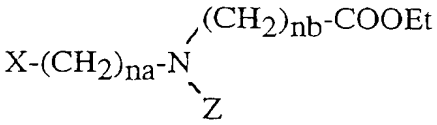
- treating compound m with HCl, preferably 3N HCl in MeOH at 50°C overnight, leading to the following compound n



m

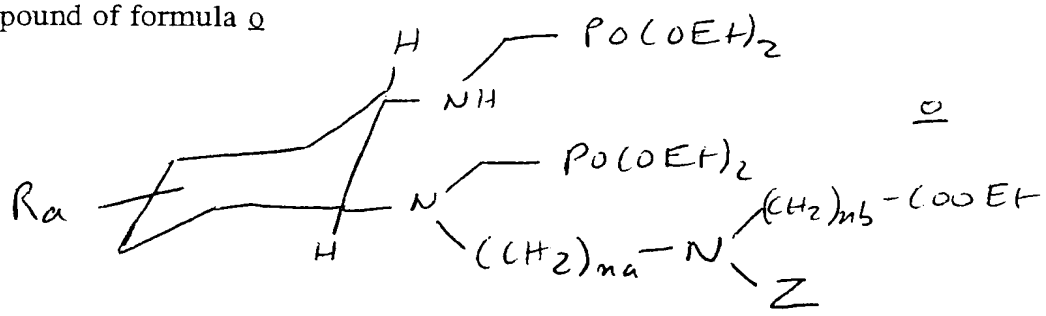
- contacting compound n :

* either with 1 equivalent of compound j



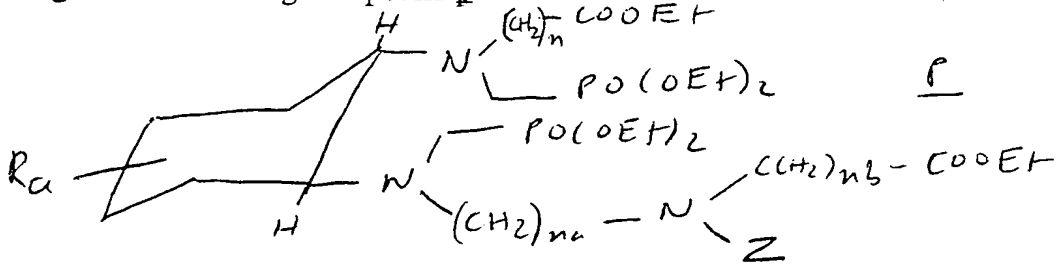
j

as described above, preferably at 70°C during 2 days, leading to the following compound of formula o



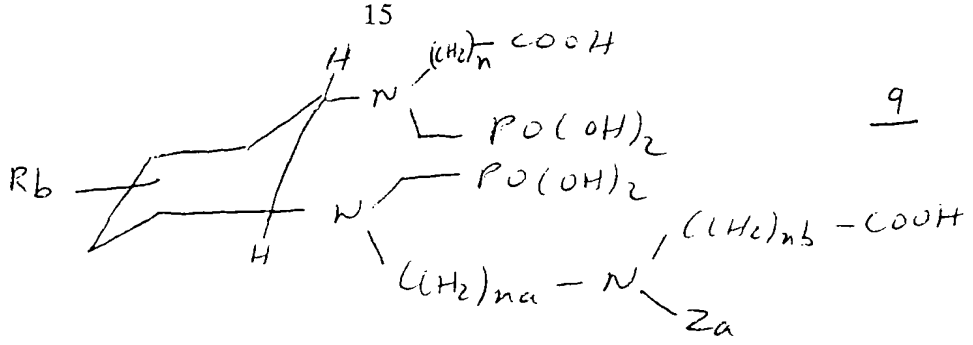
o

. contacting compound o with X-$(CH_2)_n$-COOEt, wherein X represents an halogen atom, and n represents an integer from 1 to 5, preferably at reflux during 15h, leading to the following compound p



p

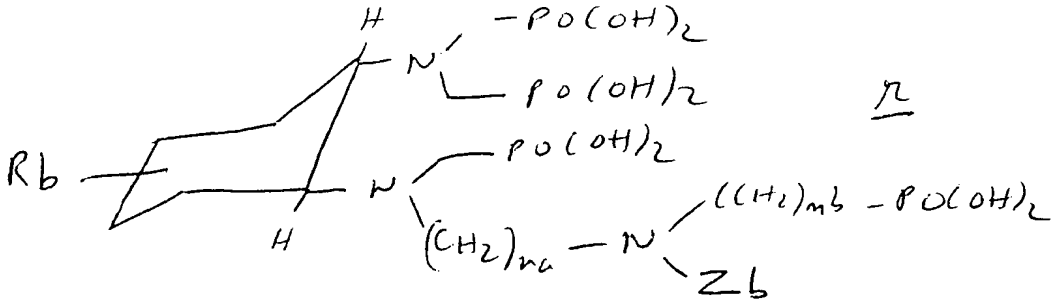
. treating compound p with HCl, preferably 6N HCl at reflux overnight, leading to the following compound q



q

wherein Za represents H or -$(CH_2)_{nb}$-COOH, na, nb and n being such as defined above, $R_b$ represents H or $NH_2$,

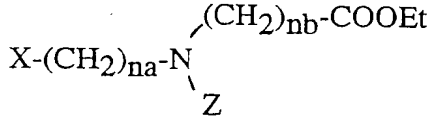
. if desired, treating compound q with phosphorous acid, preferably by stirring 30 mn at 80°C, leading to the following compound r



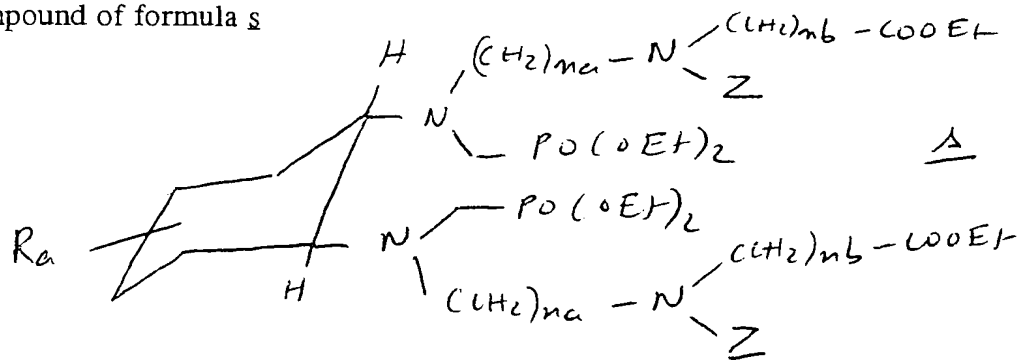
r

wherein Zb represents H or -$(CH_2)_{nb}$-$PO(OH)_2$

* or with 2 equivalents of compound j

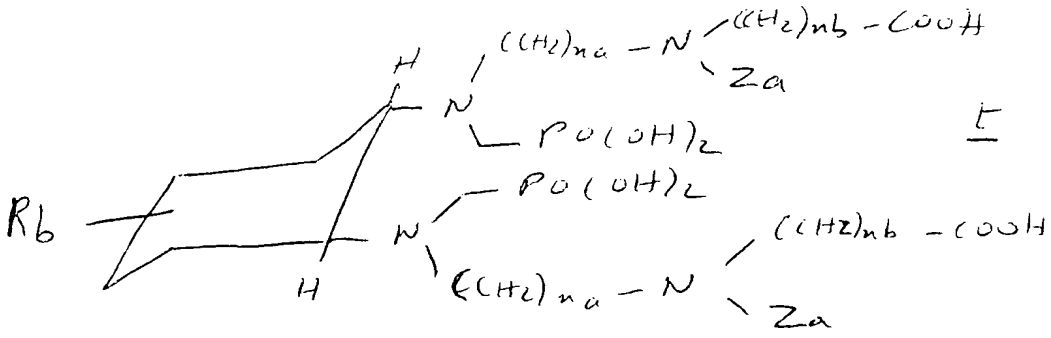


as described above, preferably at 70°C during 2 days, leading to the following compound of formula s



s

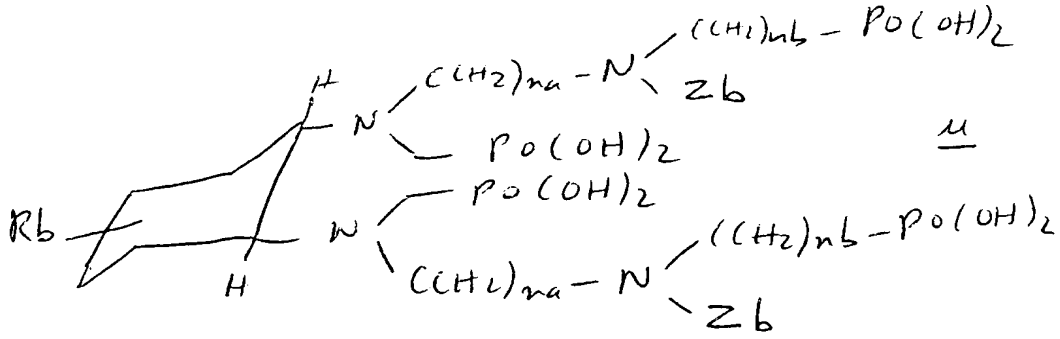
. treating compound s with HCl, preferably 6N HCl at reflux overnight, leading to the following compound t



t

wherein Za represents H or -$(CH_2)_{nb}$-COOH, na, nb and n being such as defined above, $R_b$ represents H or $NH_2$,

. if desired, treating compound t with phosphorous acid, preferably by stirring 30 mn at 80°C, leading to the following compound u



u

wherein Zb represents H or -$(CH_2)_{nb}$-$PO(OH)_2$.

Compound of formula a can be obtained according to the method described in Gestin et al., 1997, and Loussouarn et al., 1998.

Compounds wherein Rb represents $NH_2$ obtained according to the processes described above, can then be transformed in order to correspond to compounds of formula (I) wherein R represents a group carrying a function liable to bind, if necessary via a binding site, to molecules as defined above.

By way of example, compounds of formula (I) wherein R represents -N=C=S, can be obtained by treatment of said compounds wherein Rb represents $NH_2$ with CSCl2, preferably in acidic or basic conditions.

Compounds of formula (I) wherein R represents a group carrying a function linked, if necessary via a binding site, to molecules as defined above,

can then be obtained by coupling said compounds, wherein R represents a group carrying a function liable to bind to said molecules, with said molecules.

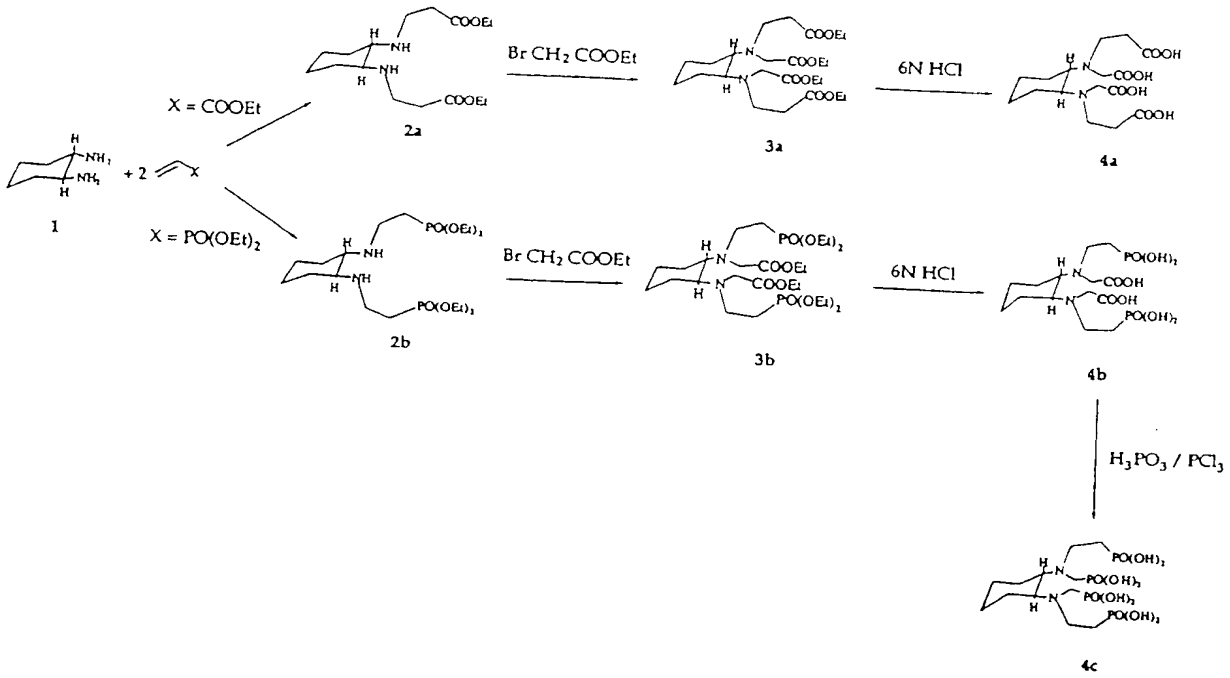
Complexes according to the invention are advantageously obtained by incubating the compounds withe the radioelements at 37°C during 3 hours.

The invention will be further illustrated in the following examples for the preparation of compounds and complexes according to the invention.

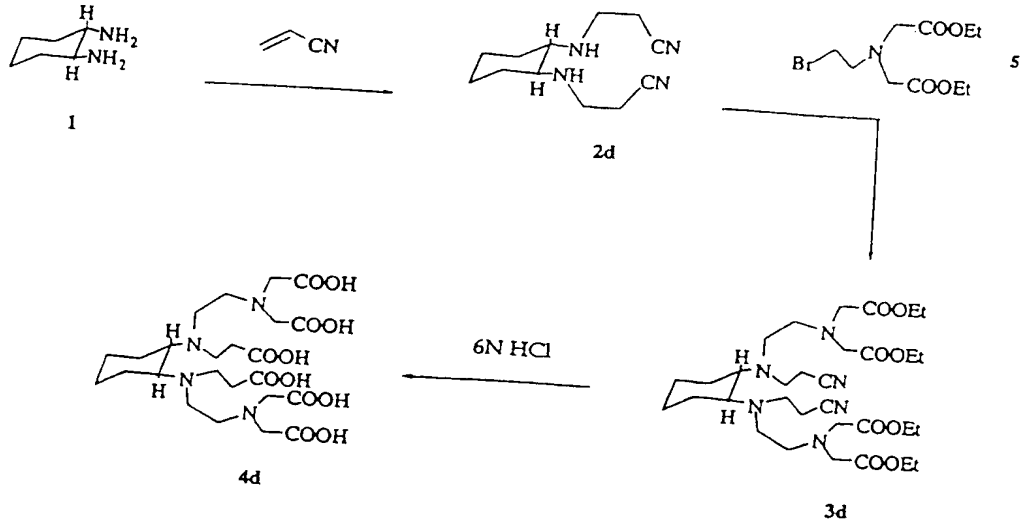
In order to save the functionalized reactional intermediate, i.e. the (1*R**, 2*R**, 4*S**)-4-acetamido-1,2-diaminocyclohexane dihydro chloride compound, the commercial product, *trans*-1,2- diaminocyclohexane **1**, has been used as starting material.

Two approach routes to differently substituted amines were carried out successfully.

- The first depicted in schemes I and II was the Michael type addition of primary amines to some vinylic derivatives to provide monoaddition with high selectivity (Bergeron et al., 1981), allowing N-alkylation to be envisaged at this step. In strategy depicted in scheme I, compounds **2a** and **2b** were alkylated by ethyl bromoacetate under conditions recommanded by Studer (Studer and Meares, 1992) (KI and $Na_2CO_3$) to give tetraesters **3a** and **3b**. Acid-catalysed hydrolysis of the ester functions was performed in 3M hydrochloric acid to give the tetracarboxylic acid **4a** and the mixed acid **4b**. At last, in order to generate the structure **4c**, carboxylic functions were converted into phosphonic functions using $H_3PO_3/PCl_3$ according to the method of Krüger and Bauer (Krüger and Bauer, 1972). The other strategy described in scheme II required preparation of protected bis-carboxymethylated amino ethyl bromide. In view to convenience of deprotecting ethyl esters by acid-catalysed hydrolysis, *N,N-bis*(ethylacetate)-2-bromoethyl-amine was prepared according to the Williams and Rapoport's procedure (Williams and Rapoport, 1994) with minor modifications. N-alkylation of **2d** with the branching group in a mixed solvent system ($CH_3CN$/EtOH) at 70°C gave **3d** in 60% yield. Acid-catalysed hydrolysis of the ester functions as well as nitriles is the more convenient method (Ornstein et al., 1989), of hexacarboxylic acid **4d** preparation.

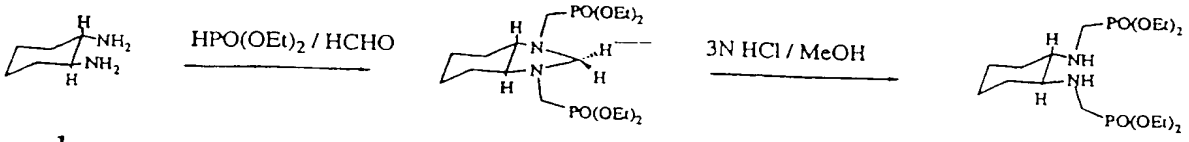



scheme I




scheme II

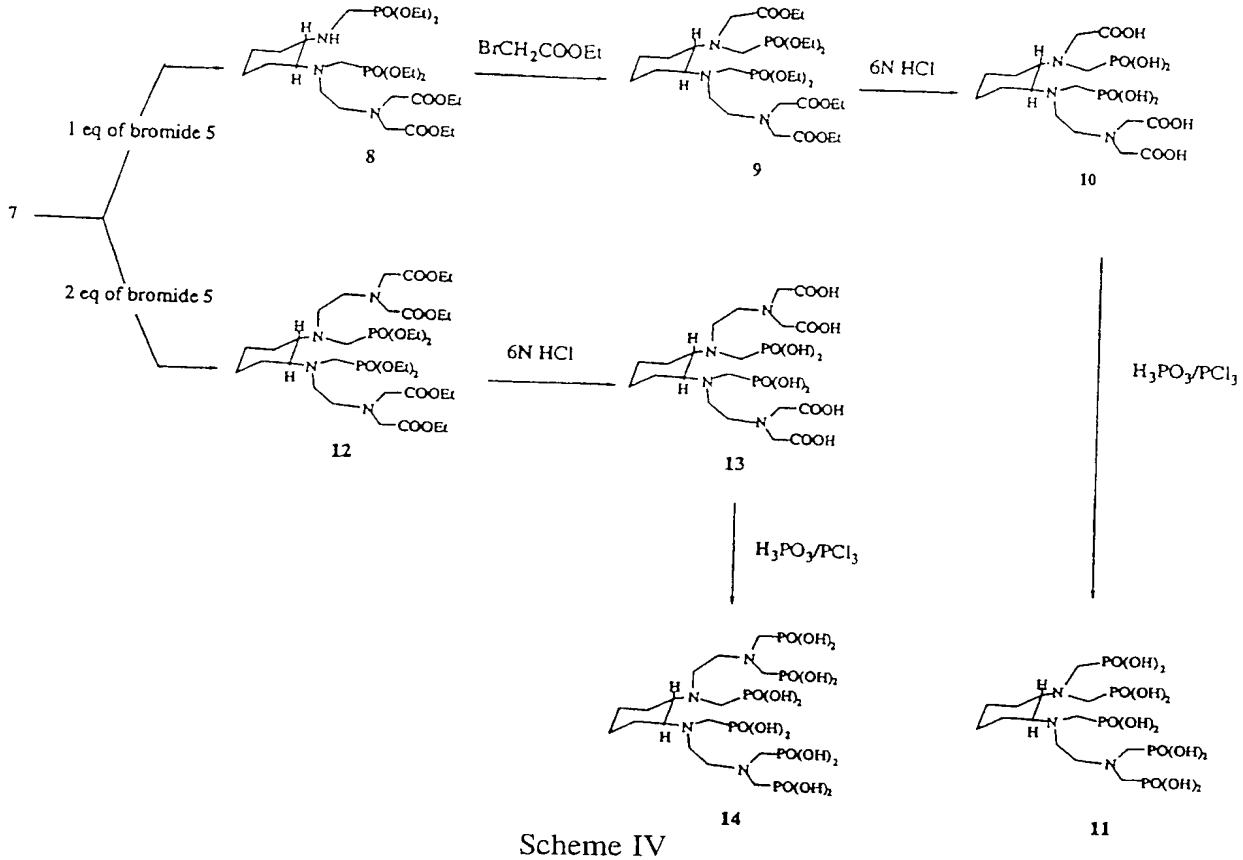
- In the case of certain diamines as *trans*-1,2-diaminocyclohexane **1**, the second route, as shown in schemes III, IV allowed the aminophosphonomethylation of amines protected by a methylene bridge between the two nitrogen atoms of **1**. This protecting group will subsequently provide for a different functionalization on the amine. The reaction of Kabachnick-field described and detailed by Baily and Burgada (Baily and Burgada, 1995), gave compound **6** which was prepared from paraformaldehyde and diethylphosphite in THF. **7** was obtained by removing the protecting group in acidic conditions.




**1** **6** **7**

scheme III

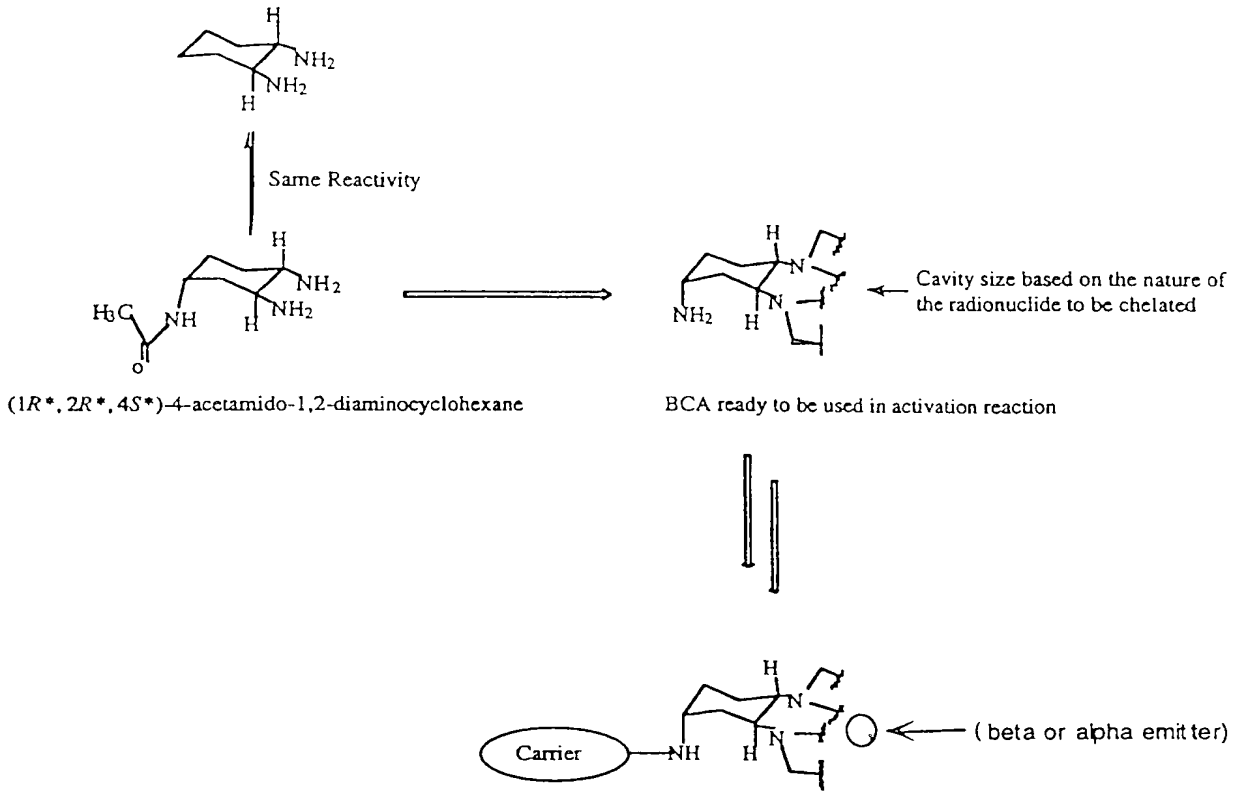
The monoalkylation or the dialkylation (see scheme IV), depending on the stoechiometry of the reaction gave respectively compounds **8** and **12**. The mixed acid **13** was obtained after hydrolysis of **12** in 6M hydrochloric acid and the hexaphosphonic acid **14** was prepared according to the method of Bauer and Kruger as described above. The synthesis of chelating agents **10** and **11** required an additional step which was the alkylation of **8** by ethyl bromoacetate to give the mixed ester **9**. Acid-catalysed hydrolysis gave **10** and **11** after reaction of conversion described all above.




Scheme IV

In conclusion, different non-functionalized ligands bearing aminophosphonate or aminocarboxylate chelate groups and mixed chelate groups were prepared and tested for their complexation properties with $^{153}Sm$. The synthetic method described above was applied to the previously synthesized intermediate, the (1*R**, 2*R**, 4*S**)-4-acetamido-1,2-diaminocyclohexane, resulting in the synthesis of several polyaminocarboxylic acids, polyaminophosphonic acids and mixed semi-rigid functionalized ligands (BCA).

The different access routes to non-functionalized compounds described here were used without modifying the synthesis in order to obtain their functionalized homologues ready to be used in a coupling reaction as described in scheme V. We observed the influence of aminocarboxylic acid and aminophosphonic acid functions on the stability of the resulting complexes.




Scheme V

## Experimental

### *General Procedures*

All experiments were performed under nitrogen. Solvents were distilled prior to reactions. The primary chemicals used were commercial products (Sigma-Aldrich Company). Product purity and reaction progress were monitored on thin-layer chromatography (TLC) plates (60 F254, Merck), and liquid chromatography was carried out on a silica gel column (Merck 60,70-230 mesh). TLC revelation was performed under UV light (254 nm) or by iodine.

*Nuclear Magnetic Resonance (MNR)*

$^{1}H$ and $^{13}C$ NMR spectra were recorded on a Bruker AC 250 spectrometer (250 Mhz). Chemical shifts are reported in ppm to phosphoric acid as reference (85% $H_3PO_4$ in heavy water), positive values being downfield.

Chemical shifts (d) are reported in ppm. Coupling constant J is reported in Hertz (Hz).

*Mass Spectrometry (MS)*

MS spectra were recorded on a Mat Finnigan LCQ Ion Trap mass apparatus using the electrospray method in negative or positive mode.

*Starting Material*

The bisphosphonate **6** was prepared in our laboratory according the synthesis procedure of Baily and Burgada with minor modifications.

*Synthesis and Specstroscopic data*

***N,N'*-[(2-ethoxycarbonyl)eth-1-yl]-*trans*-cyclohexane-1,2-diamine 2a:** To freshly distilled *trans*-1,2-diaminocyclohexane 1 (1 ml, 8.33 mmol) in 50 ml of ethanol was added vinyl propionate (1.50 ml, 13.7 mmol) in one portion. After stirring 20h at room temperature, the reaction mixture was concentrated by rotary evaporation to yield a pale yellow oil (2.6 g, 8.32 mmol, 100%) witch was used directly in the next step. $^{1}H$ NMR ($CDCl_3$): d 1.22 (t, 12H), 1.67 (m, 2H), 1.82 (m, 2H), 2.06 (m, 2H+2H), 2.43 (t, 4H), 2.67 (dt, 2H), 2.98 (dt, 2H), 4.10 (q, 4H). $^{13}C$ NMR ($CDCl_3$): d 14.17, 24.31, 31.46, 35.34, 42.19, 60.23, 61.29, 172.69 . (M+H+): 315

***N,N'*-[(2-diethylphosphono)eth-1-yl]-*trans*-cyclohexane-1,2-diamine 2b:** To freshly distilled *trans*-1,2-diaminocyclohexane 1 (1 ml, 8.33 mmol) in 50 ml of ethanol was added diethyl vinyl phosphonate (2.80 ml, 18.21 mmol). The reaction mixture was allowed to stir at reflux during 15 hours. After removal the solvent under reduced pressure, the resulting oil was purified by column chromatography (silica gel, $CH_2Cl_2$-EtOH 1:1) to give 2.9 g of a limpid oil (6.65 mmol, 80%). $^{1}H$ NMR ($CDCl_3$): d 0.94 (m, 2H), 1.15 (m, 2H), 1.24 (t, 12H), 1.64 (m, 2H), 1.83-1.95 (m, 8H), 2.05 (m, 2H), 2.71 (dt, 2H), 2.97 (dt,

2H), 4.07 (dq, 8H). 13C NMR ($CDCl_3$): d 16.37, 16.46, 25.42 ($J_{C-P}$ : 149 Hz), 28.20, 31.41, 40.47, 40.51, 61.29, 61.40, 61.50. (M+H+): 443

*N,N'*-[(2-cyano)eth-1-yl]-*trans*-cyclohexane-1,2-diamine **2d:** To freshly distilled *trans*-1,2-diaminocyclohexane 1 (1 ml, 8.33 mmol) in 50 ml of ethanol was added acrylonitryle (1.20 ml, 18.32 mmol). After stirring 20h at room temperature, the reaction mixture was concentrated by rotary evaporation to yield an pale yellow oil witch was purified by recristallisation in diethylether to give 1.40 g of a white solid (6.35 mmol, 78%): mp : 65°C

1H NMR ($CDCl_3$): d 1.02 (m, 2H), 1.22 (m, 2H), 1.70-1.79 (m, 2H + 2H), 2.02-2.17 (m, 2H+2H), 2.49 (t, 4H), 2.80 (dt, 2H), 3.02 (dt, 2H). 13C NMR ($CDCl_3$): d (M+H+): 221

*N,N'*-[(2-ethoxycarbonyl)eth-1-yl]-*N,N'*-(ethylacetate)-*trans*-cyclohexane-1,2-diamine **3a:** To a solution of 2a (1 g; 3,.18 mmol) in 50 ml of freshly distilled CH3CN under nitrogen were added $Na_2CO_3$ (0.50 g; 3.01 mmol) and KI ( g; mmol). After stirring for 1 hour at 60°C, $BrCH_2COOEt$ (1.80 ml; 7.15 mmol) was added dropwise. The reaction mixture was kept at this temperature over a period of 24 hours prior to cooling to room temperature, filtration and concentration under reduced pressure. The residue was taken up in $CHCl_3$ (200 ml) and washed with water. The organic layer was dried ($Na_2SO_4$) and concentrated under reduced pressure to give a yellow-brown oil. The crude product was purified by column chromatography (silica gel, $CH_2Cl_2$-EtOH 95:5). The fractions containing pure product were collected and dried to give an limpid oil (0.8 g; 1.56 mmol; 49 %). 1H NMR ($CDCl_3$): d 1.10 (m, 4H), 1.26 (t, 12H), 1.91 (m, 2H), 2.00 (m, 2H), 2.51 (dt+m, 4H+2H(CH cycle)), 2.95 (dt, 4H), 3.39 (d, 4H), 4.12 (m, 8H). (M+H+): 515

*N,N'*-(ethylacetate)-*N,N'*-[(2-diethylphosphono)eth-1-yl]-*trans*-cyclohexane-1,2-diamine **3b:** The tetraester has been prepared as described above for compound from 1 g of compound 2b, $Na_2CO_3$ (0.70g, 6.60 mmol), KI (0.40, 2.40 mmol) and ethylbromoacetate (1.80 ml; 7.15 mmol). Purification by chromatography ($SiO_2$ ($CH_2Cl_2$-MeOH 99 : 1) gave 0.58 g of a pale yellow oil (0.94 mmol; 41%).1H NMR ($CDCl_3$): d 1.11 (m, 4H), 1.25 (t, 6H), 1.29 (t, 12H), 1.70 (m, 2H), 1.85-2.10 (m, 4H+2H), 2.90 (t, 4H), 3.38 (d, 4H), 4.09 (m, 12H). 13C NMR ($CDCl_3$): d 14.11, 16.31, 16.41, 25.63, 26.03 ($J_{C-P}$ : 135), 27.98, 44.86, 52.43, 60.26, 61.31, 61.41, 63.08, 172.46. (M+H+): 615

**General procedure for preparation of corresponding acids: *trans*-cyclohexane-1,2-diamine-*N*,*N'*-acetic-*N*,*N'*-propionic acid 4a and *trans*-cyclohexane-1,2-diamine-*N*,*N'*-acetic-*N*,*N'*-ethylphosphonic acid 4b:**

Compound 3a or 3b (1 g) was dissolved in 6N aqueous hydrochloric acid (12ml) and heated to reflux overnight. The refrigerant was removed, and the reaction mixture was kept at 70°C to dryness. An additional aqueous hydrochloric acid 6N (12 ml) was then added, and the solution was heated to dryness. the residue was taken up in MeOH and evaporated under reduced pressure. This step repeated twice gave the corresponding acid as an off-white solid, which was dried under vacuum and kept under nitrogen.

**Compound 4a:** $^1$H NMR ($D_2O$): d 1.25-1.40 (m, 4H), 1.60-2.15 (m, 4H), 2.28 (m, 2H), 2.75 (t, 2H), 2.96 (t, 2H), 3.22 (m, 2H), 3.50-3.90 (m, 4H), 4.15 (s, 1H), 4.28 (s, 1H) $^{13}$C NMR ($CDCl_3$): d 25.66, 26.22, 28.87, 31.20, 31.99, 47.25, 54.93, 66.92, 175.03, 176.46 (M-H+): 373

**Compound 4b:** $^1$H NMR ($D_2O$): d 1.05-1.40 (m, 4H), 1.65-2.15 (m, 4H), 2.90-3.25 (m, 6H), 3.45-3.65(m, 2H), 3.70-3.95 (m, 2H). $^{13}$C NMR ($CDCl_3$): d 25.66, 26.22, 28.87, 31.20, 31.99, 47.25, 54.93, 66.33, 176.73 (M-H+): 445

***trans*-cyclohexane-1,2-diamine-*N*,*N'*-ethylphosphonic-*N*,*N'*-methylphosphonic acid 4c:** A mixture of compound 4c (0.5 g; 1.12 mmol) and phosphorous acid (0.202 g; 2.46 mmol) in 10 ml of dry toluene was heated to 80°C and stirred for 30 min. $PCl_3$ ( 0.22 ml; 2.46 mmol) was then added dropwise, and the reaction mixture was kept at this temperature for 20 hours before being cooled to room temperature. The solvent was discarded and the residual product dissolved in a small volume of water. After filtration, the filtrate was evaporated to give a residue which was purified by precipitation in warm acetone and collected by filtration. The purification step was repeated twice to give 4c which was dried under vacuum and kept under nitrogen (0.460 g; 0.83 mmol; 74%). $^1$H NMR ($D_2O$): d 1.15-1.65 (m, 4H), 1.75-2.10 (m, 2H), 2.15-2.40 (m, 6H), 3.00-3.60 (m, 10H). $^{13}$C NMR ($CDCl_3$): d (M-H+): 517.

***N*,*N*-*Bis*(ethylacetate)-2-bromoethyl-amine 5:** Bromide 5 was synthesised in our laboratory according the synthesis procedure of Williams and Rapoport with minor modifications concerning the bis N-alkylated ethanolamine synthesis. To a 4°C solution of ethanolamine (6ml; 0.1 mol) in 100 ml of dried acetonitrile was added dropwise ethylbromoacetate (7.4 ml; 66 mmol) over a period of 20

min during which time a large quantity of precipitate formed. The mixture was allowed to stir for 2 hours at this temperature. The white solid was removed by filtration and washed with a small quantity of acetonitrile. The filtrate was concentrated under reduced pressure. The resulting liquid was taken up in $CHCl_3$ (100mL) and washed with water. The organic layer was dried ($Na_2SO_4$) and concentrated under reduced pressure to give a liquid which was used directly in the next step (5.9 g; 25.32 mmol; 77%). The dialkylated ethanolamine and $Ph_3P$ (7.72 g; 27.9 mmol) were dissolved in $CH_2Cl_2$ (100mL). The mixture was cooled in an ice bath and vigorously stirred while NBS (4.96 g; 27.9 mmol) was added in small portions. After the solution was stirred at 0°C for two hours, evaporation of the solvent gave a semisolid which was tritured with ether and the resulting solid was separated by filtration. the filtrate was evaporated to give an oil which was purified by column chromatography (silica gel, $CH_3Cl$). (6.14 g; 20.7 mmol; 62% overall) $^1H$ NMR ($CDCl_3$): d 1.26 (m, 6H), 3.15 (t, 2H, J = 7.75 Hz), 3.44 (t, 2H, J = 7.75 Hz), 3.59 (s, 4H), 4.15 (q, 4H). $^{13}C$ NMR ($CDCl_3$): d (M+H+): 297

***N,N'*-[(2-cyano)eth-1-yl]-N,N'-[*N'',N''*-*bis*-(ethylacetate-2-aminoethyl)]-*trans*-cyclohexane-1,2-diamine 3d:** To a solution of compound 2d (1 g; 4.54 mmol) and bromide 5 (3 g; 10.13 mmol) in a mixed solvent system ($CH_3CN$-EtOH, 1:1) was added $Na_2CO_3$ (1.4 g; 13.20 mmol) and KI (0.75 g; 4.54 mmol). After stirring for 2 days at 70°C, the reaction mixture was filtered and concentrated under reduced pressure. The residue was taken up in $CHCl_3$ (200 ml) and washed with water. The organic layer was dried ($Na_2SO_4$) and concentrated under reduced pressure to give a yellow-brown oil. The crude product was purified by column chromatography (silica gel, $CH_2Cl_2$-EtOH 98:2). The fractions containing pure product were collected and dried to give an limpid oil (1.09 g; 1.68 mmol; 37%).$^1H$ NMR ($CDCl_3$): d 1.13 (m, 4H), 1.27 (t, 12H), 1.73 (m, 2H), 1.86 (m, 2H), 2.30-2.85 (m, 4H+2H), 2.95 (m, 2H), 3.55 (s, 8H), 4.17 (m, 12H). $^{13}C$ NMR ($CDCl_3$): d 14.25, 18.63, 25.79, 27.24, 47.35, 48.98, 53.78, 55.43, 60.54, 62.64, 119.49, 171.11. (M+H+): 652

***trans*-cyclohexane-1,2-diamine-*N,N'*-propionic-*N,N'*-[*N'',N''*-*bis*-(2-aminoethyl)]-tetra-acetic acid 4d:** this hexaacid has been prepared as described above for compounds 4a & 4b from 1 g of the ester 3d and two volumes of 20 ml HCl (6N). $^1H$ NMR ($D_2O$): 1.20-2.00 (m, 10 H), 2.30 (m, 2H), 2.40-3.00 (m, 4H), 3.10-3.95 (m, 14 H), 4.20 (m, 4H) d $^{13}C$ NMR ($D_2O$) : d 26.89, 30.49, 40.99, 52.00, 55.18, 55.47, 58.97, 165.50, 170.38. (M-H+): 547

***N,N'*-(diethylphosphono-methyl)-*trans*-cyclohexane-1,2-diamine 7:** was synthesised in our laboratory according the synthesis procedure of Baily and Burgada with minor modifications. Freshly distilled *trans*-1,2-diaminocyclohexane 1 (3.6 ml, 30 mmol) and diethyl phosphite (7.24 ml, 60 mmol) were dissolved in THF (40ml). The mixture was stirred at reflux and paraformaldehyde (2.8 g, 93 mmol) was added over a 30-min period and the reaction mixture was stirred at reflux for 4 hours. The solvent was evaporated to afford a residue which was taken up in $CHCl_3$. The organic layer was washed with brine (2*100 ml), dried and evaporated to leave a crude oil. A purification by column chromatography (silica gel, $CH_2Cl_2$-EtOH 96:4) gave 6 (9.2 g, 21.60 mmol, 72%). 6 was then dissolved in MeOH (40 ml) and 35% HCl (15 ml) was added. The mixture was stirred at 50°C overnight. MeOH was removed. the aqueous layer was adjusted to 50 ml with $H_2O$ and then neutralized by $HNaCO_3$. bisphosphonate 7 was extracted by $CHCl_3$. Organic layers were collected, dried and evaporated to give 5.6 g of bisphosphonate 7 (13.52 mmol, 62%, 45% overall). $^1H$ NMR ($CDCl_3$): 1.10 (m, 2 H), 1.27 (t, 12H), 1.48 (m, 2H), 1.76 (m, 2 H), 2.13 (m, 2H), 2.98 (m, 2H), 3.10 (t, 2H), 3.35 (t, 2H), 4.12 (m, 8H). $^{13}C$ NMR ($CDCl_3$): d: 16.36, 16.39, 16.45, 16.38, 24,01, 28.23, 39.67 (JP-C = 156 Hz), 60.74, 60.89, 63.16, 63.23, 63.26, 63.33. ($M+H^+$): 415

***N,N'*-(diethylphosphono-methyl)-*N*-[*N'',N''*-*bis*-(ethylacetate-2-aminoethyl)]-*trans*-cyclohexane-1,2-diamine 8:** The mixed ester has been prepared in a mixed solvent system ($CH_3CN$-$H_2O$, 1:1) as described above for compound 3d from bisphosphonate 6 (1 g, 2.41 mmol) , $Na_2HPO_4$ (0.5 g; 3.50 mmol) and 1 equivalent of bromide 5 (0.71 g; 2.41 mmol). After stirring for 2 days at 70°C, the reaction mixture was filtered and concentrated under reduced pressure. The residue was taken up in $CHCl_3$ (200 ml) and washed with water. The organic layer was dried ($Na_2SO_4$) and concentrated under reduced pressure to give a yellow-brown oil witch was used directly in the next step. $^1H$ NMR: 1.00-1.20 (m, 4 H), 1.25 (t, 6H), 1.31 (t,12H), 1.72 (m, 2H), 2.04 (m, 2H), 2.23 (m, 1 H), 2.70-2.95 (m, 6H), 3.12 (m, 2H), 3.65 (s, 4H), 4.15 (m, 18H).

***N,N'*-(diethylphosphono-methyl)-*N'*-(ethylacetate)-*N*-[*N'',N''*-*bis*-(ethylacetate-2-aminoethyl)]-*trans*-cyclohexane-1,2-diamine 9:** The tetraester is prepared as described above for compounds 3a & 3b from compound 8,

$Na_2CO_3$, KI and ethylbromoacetate (1 equivalent). Purification by chromatography ($SiO_2$) gave a oil.

*N,N'*-(diethylphosphono-methyl)-*N,N'*-[*N'',N''*-*bis*-(ethylacetate-2-aminoethyl)]-*trans*-cyclohexane-1,2-diamine **12**: The mixed ester is prepared as described above for compound 3d from bisphosphonate 7 (1g, 2.41 mmol), $Na_2HPO_4$ (1 g, 7.04 mmol) and 2 equivalents of bromide 5 1.80 g, 6.08 mmol). Purification by chromatography ($SiO_2$ ($CH_2Cl_2$-MeOH 95 : 5) gave a pale yellow oil (0.59 g, 0.70 mmol, 29%). $^1H$ NMR: 1.16 (m, 4H), 1.25 (t, 12H), 1.33 (t, 12H), 1.69 (m, 2H), 1.86 (m, 2H + 2H), 2.70-3.50 (m, 8H + 2H + 2H), 3.57 (s, 8H), 4.12 (m, 16H). (M+H+): 847

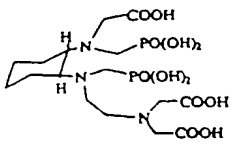
*trans*-cyclohexane-1,2-diamine-*N,N'*-methylphosphonic-*N*-*acetic*-,*N'*-[*N'',N''*-*bis*-(2-aminoethyl)]-tetra-acetic acid **10** & *trans*-cyclohexane-1,2-diamine-*N,N'*-methylphosphonic-*N,N'*-[*N'',N''*-*bis*-(2-aminoethyl)]-tetra-acetic acid **13** : Those compounds are prepared as described above for compound 4a, 4b and 4d.

**Compound 13:** $^1H$ NMR ($D_2O$): 1.26 (m, 2 H), 1.45 (m, 2H), 1.75-2.00 (m, 4H), 2.80 (m, 2H), 3.00-3.70 (m, 12H), 4.10 (br s, 8H). (M-H+): 619

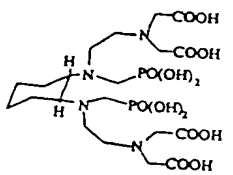
*trans*-cyclohexane-1,2-diamine-*N,N,N'*-methylphosphonic-*N'*-[*N'',N''*-*bis*-(2-aminoethyl)]-di-methylphosphonic acid **11** & *trans*-cyclohexane-1,2-diamine-*N,N'*-methylphosphonic-*N,N'*-[*N'',N''*-*bis*-(2-aminoethyl)]-tetra-methylphosphonic acid **14** : Those compounds are prepared as described above for compound 4c.

**153Sm complexation studies**

Complexation studies with Samarium 153 were performed on the two following chelating agents 10 (AL 247) and 13 (AL 245)




10




13

Radiochemistry purity was measured on ITLC-SG chromatographic profiles. Radioactivity was quantified using a Phosphorimager 445SI apparatus.

Samarium 153 was furnished under $^{153}SmCl_3$ form in HCl 0,04N with a 5,2 GBq/ml volumic activity and a 40 GBq/mg specific activity.

They were tested for their complexation properties by using an excess of 10 to 50 equivalents of chelating agent.

Competition studies were performed against EDTMP according to the following method :

- first step : 50 equivalents of one of the chelating agents and a fixed amount of 153Sm were incubated at 37°C during 3 hours in order to form the 153Sm-CA complex (CA = Chelating Agent),

- second step : 50 equivalents of EDTMP were added to the previous solution and kept 3h at 37°C in order to measure the decomplexation. Another measure was performed 72h after to ensure a complete decomplexation possibility.

Results :

| | % of non decomplexed 153Sm-CA | |
|---|---|---|
| chelating agent | after 3h | after 72h |
| 10 (AL 247) | 100 | 67 |
| 13 (AL 245) | 100 | 100 |

AL 247 and AL 245 present very good complexation properties for 153Sm and in any cases better than EDTMP

Furthermore, stability was performed on AL 245 in human serum media at 37°C at different time and showed no loss of 153Sm from AL 245 at either 24, 48, 72 and 96h.

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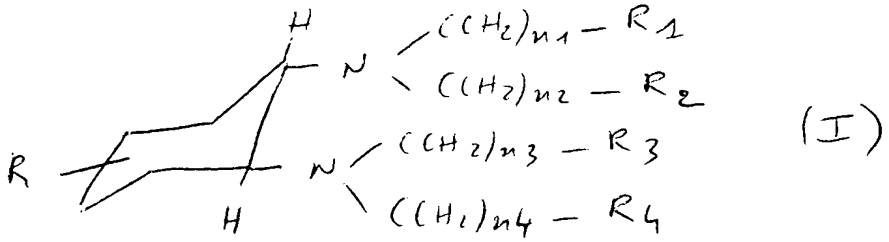
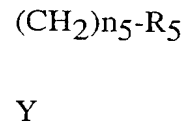
# CLAIMS

1. Compounds of the following formula (I) :



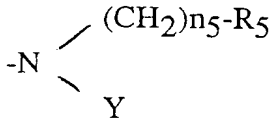
(I)

in which :

- $n_1$, $n_2$, $n_3$ and $n_4$, independently from each other, represent an integer from 1 to 5, preferably from 1 to 3,
- $R_1$, $R_2$, $R_3$ and $R_4$, independently from each other, represent :
  . -COOH,
  . $-PO(OH)_2$,
  . $-N\begin{  $

wherein $n_5$ represents an integer from 1 to 5, preferably from 1 to 3, $R_5$ represents -COOH or $-PO(OH)_2$, and Y represents H or a group $-(CH2)n_6-R_6$ in which $n_6$ represents an integer from 1 to 5, preferably from 1 to 3, and $R_6$ represents -COOH or $-PO(OH)_2$,

provided that at least one of $R_1$, $R_2$, $R_3$ or $R_4$ represents a group

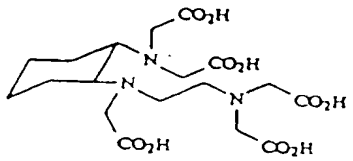


such as defined above,

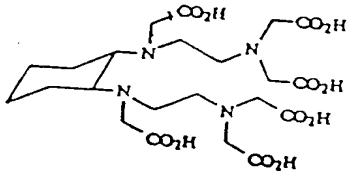
- R represents :
  . H, or $-NHCOCH_3$, or
  . a group carrying a function liable to bind, if necessary via a binding site, to molecules, such as antibodies, haptens or peptides, which are able to bind specifically with epitopes located at the surface of the cells of the organism, or to chemical or biological compounds located at the surface of a solid carrier, or

. a group carrying a function linked, if necessary via a binding site, to molecules as defined above,

the two following compounds, CDTPA and CTTHA, being excluded :



CDTPA

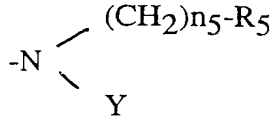


CTTHA

2. Compounds according to claim 1, characterized in that :

- when $R_1$, $R_2$, $R_3$ or $R_4$ represents -COOH or -$PO(OH)_2$, then $n_1$, $n_2$, $n_3$ or $n_4$ represents 1 respectively,

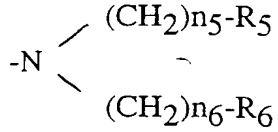
- when $R_1$, $R_2$, $R_3$ or $R_4$ represents a group



then $n_1$, $n_2$, $n_3$ or $n_4$ represents 2 or 3 respectively, and preferably 2,

- $n_5$, and optionally $n_6$, represents 1.

3. Compounds according to claims 1 or 2, characterized in that at least one, and more preferably two of $R_1$, $R_2$, $R_3$ and $R_4$, represent a group



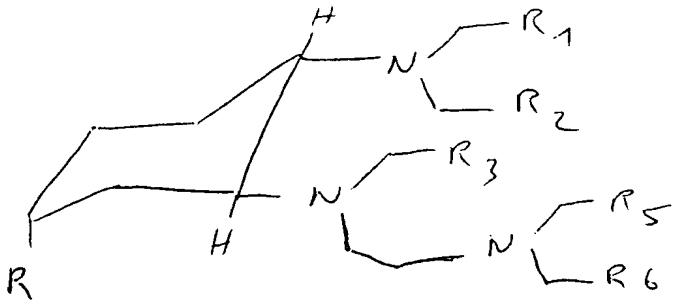
wherein $n_5$, $n_6$, $R_5$ and $R_6$ are defined in claims 1 or2.

4. Compounds according to anyone of claims 1 to 3, characterized in that R represents a group carrying a function liable to bind, if necessary via a binding site, to molecules, such as antibodies, haptens or peptides, as defined in

claim 1, and in particular R represents a group chosen among all the coupling functions for vector or solid support binding.

5. Compounds according to anyone of claims 1 to 3, characterized in that R represents a group carrying a function linked, if necessary via a binding site, to molecules, such as antibodies, haptens or peptides, as defined in claim 1.

6. Compounds according to anyone of claims 1 to 5 of the following formula (III) :



(III)

in which $R_1$, $R_2$, $R_3$, $R_5$ and $R_6$ independently from each other represent -COOH or -$PO(OH)_2$, and R is a group as defined in claims 2 to 5.

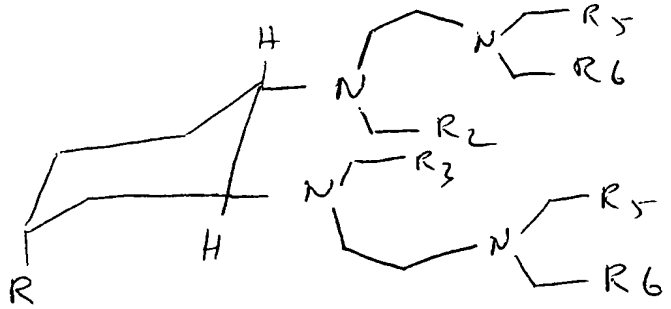
7. Compounds according to claim 6, of formula (III) wherein :

. $R_1 = R_5 = R_6 = COOH$ and $R_2, = R_3 = PO(HO)_2$, or

. $R_1 = R_2 = R_3 = R_5 = R_6 = COOH$, or

. $R_1 = R_2 = R_3 = R_5 = R_6 = PO(OH)_2$.

8. Compounds according to anyone of claims 1 to 5, of the following formula (IV) :



(IV)

wherein $R_2$, $R_5$ and $R_6$, independently form each other, represent -COOH or -$PO(OH)_2$, and R is a group as defined in claims 2 to 5.

9. Compounds according to claim 8 of formula (IV) wherein :

. $R_2 = R_3 = PO(OH)_2$, and $R_5 = R_6 = COOH$, or

. $R_2 = R_3 = R_5 = R_6 = COOH$, or

. $R_2 = R_3 = R_5 = R_6 = PO(OH)_2$.

10. Complexes between a compound according to anyone of claims 1 to 9, and a radioactive element.

11. Complexes according to claim 10, characterized in that said radioelements are $\alpha$ or $\beta$ emitter radiometals.

12. Complexes according to claim 11, characterized in that the compound is chosen among those defined in anyone of claims 5 to 9, and more particularly among those compounds wherein the group R comprises :

- an antibody (polyclonal or monoclonal) liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism,
- or an hapten, i.e. a non-immunogenic molecule of low MW capable of inducing the production of antibodies against itself, said hapten being liable to recognize and to bind to one or several molecules already bound, in a first step of the treatment, to epitopes on the surface of specific cells in the organism,
- or a peptide resulting from the association of different amino acids and liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism.

13. Use of a complex according to claims 11 or 12, for the manufacture of a medicament for radioimmunotherapy, such as for the treatment of cancers, and more particularly for the treatment against metastase proliferation.

14. Pharmaceutical compositions characterized in that they comprise an effective amount of a complex according to claims 11 or 12, in association with a suitable pharmaceutical carrier.

15. Complexes according to claim 10, characterized in that the radioelements are $\gamma$ emitter radiometals.

16. Complexes according to claim 15, characterized in that the compound is chosen among those defined in anyone of claims 5 to 9, and more particularly among those compounds wherein the group R comprises :

- an antibody (polyclonal or monoclonal) liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism,

- or an hapten, i.e. a non-immunogenic molecule of low MW capable of inducing the production of antibodies against itself, said hapten being liable to recognize and to bind to one or several molecules already bound (in a first step of the method of diagnosis) to epitopes on the surface of specific cells in the organism,

- or a peptide resulting from the association of different amino acids and liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism.

**17.** Use of a complex according to claims 15 or 16, for carrying out diagnosis methods such as radioimmunoscintigraphy.

**18.** Use of a compound of formula (I) as defined in claim 1 to 9, included compounds CDTPA and CTTHA, for :

- the manufacture of a medicament useful as antalgic, or for the treatment of pathologies where ionic imbalances occur, or against the formation of stones in the organism,

- carrying out a process for the detoxication of polluted medium, such as liquid phases polluted by bivalent or trivalent metals radioactives or not,

- carrying out a process for the radionuclides purification, said compound being bound to a solid phase,

- carrying out a bone scintigraphy, in particular in the frame of the diagnosis of osteoarticular pathology, particularly in bone cancer extension balance.

# INTERNATIONAL SEARCH REPORT

...al Application No
PCT/EP 99/08031

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07F9/38 C07C229/16 A61K51/10 A61K51/08

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07F C07C A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | US 5 292 938 A (RONNIE C. MEASE)<br>8 March 1994 (1994-03-08)<br>the whole document<br>—<br>-/— | 1-18 |

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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| 21 December 1999 | 11/01/2000 |

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category ° | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
|---|---|---|
| Y | LOUSSOUARN A ET AL: "Synthesis of new bifunctional chelating agents: (1R*,2R*,4S*)-4-isothiocyanatocyclohexane-1,2-diamine-N,N,N',N'-tetra kismethanephosphonic acid (4-ICMP) and (1R*,2R*,4S*)-4-isothiocyanato cyclohexane-1,2-diamine-N,N,N',N'-tetrakis ethanephosphonic acid (4-ICEP)" J. CHEM. SOC., PERKIN TRANS. 1 (JCPRB4,0300922X);1998; (2); PP.237-242, - 21 January 1998 (1998-01-21) XP002097516 Institut de Biologie;INSERM U.463 (ex-U.211); Nantes; 44035; Fr. (FR) cited in the application the whole document | 1-18 |
| Y | BRECHBIEL M W ET AL: "Preparation of the Novel Chelating Agent N-(2-Aminoethyl)-trans-1,2 -diaminocyclohexane-N,N',N''-pentaacetic Acid (H5CyDTPA), a Preorganized Analog of Diethylenetriaminepentaacetic Acid (H5DTPA), and the Structures of BiIII(CyDTPA)2- and BiIII(H2DTPA) Complexes" INORG. CHEM. (INOCAJ,00201669); VOL.35 (21); PP.6343-6348, - 9 October 1996 (1996-10-09) XP002097517 National Institutes of Health;Chemistry Section; Bethesda; 20892; MD; USA (US) the whole document | 1-18 |
| Y | US 5 089 663 A (RONNIE C. MEASE) 18 February 1992 (1992-02-18) the whole document | 1-18 |
| P,X | LOUSSOUARN A ET AL: "Simple and general procedure for the synthesis of semi-rigid chelating agents for radiometal complexation studies and its application to semi-rigid functionalised ligands (BCA) synthesis" MATER. SCI. FORUM (MSFOEP,02555476);1999; VOL.315-317 (RARE EARTHS '98); PP.262-267, XP000865647 Institut Biologie;Nantes; F-44035; Fr. (FR) the whole document | 1-18 |

# INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter[illegible]al Application No
PCT/EP 99/08031

| Patent document cited in search report | Publication date | Patent family member(s) | Publication date |
|---|---|---|---|
| US 5292938 A | 08-03-1994 | US 5635157 A | 03-06-1997 |
| US 5089663 A | 18-02-1992 | US 5021571 A | 04-06-1991 |
| | | US 5334729 A | 02-08-1994 |